

Amendments to the Claims

Claims 1-53 (Cancelled)

Claim 54 (Currently Amended): A type I polyketide synthase which produces a polyketide and which comprises a loading module and a plurality of extension modules, wherein:

a) said loading module loads an optionally substituted malonyl and then effects decarboxylation of the loaded moiety to provide a corresponding optionally substituted acetyl moiety for transfer to the first of said extension modules; and

b) said loading module is of the form:

(engineered-KSq)-(AT)-(ACP), wherein:

i) ACP is an acyl carrier protein domain;  
ii) AT is an acyltransferase domain which loads an optionally substituted malonyl; and

iii) engineered-KSq is a ketosynthase (KS) domain which ~~has been genetically engineered to effects~~ ~~decarboxylation of a loaded optionally substituted malonyl by mutating the active site cysteine residue to a glutamine residue, wherein said engineered-KSq domain is obtained by replacing the active site cysteine of a KS domain of an extension module with a glutamine; and~~

e) at least the first of said extension modules is not naturally associated with said loading module;

wherein the polyketide produced by the polyketide synthase is other than a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

Claim 55 (Previously Presented): A type I polyketide synthase according to claim 54, wherein said acyltransferase domain has an arginine residue in the active site.

Claim 56 (Previously Presented): A type I polyketide synthase according to claim 55, wherein said acyltransferase domain is a natural extension module acyltransferase domain.

Claim 57 (Previously Presented): A type I polyketide synthase according to claim 54, wherein the engineered-KS<sub>Q</sub> and acyltransferase domain pair produced by mutation occur together in an extension module in their unaltered state.

Claim 58 (Previously Presented): A type I polyketide synthase according to claim 55, wherein said acyltransferase domain is specific for loading with malonyl.

Claim 59 (Previously Presented): A type I polyketide synthase according to claim 55, wherein said acyltransferase domain is specific for loading with methylmalonyl.

Claim 60 (Currently Amended): A type I polyketide synthase according to claim 55, wherein said acyltransferase domain is selected from the group consisting of the acyltransferase domain of extension module 5 of the monensin polyketide synthase and the acyltransferase domain of extension module 5 of the spiramycin polyketide synthase.

Claim 61 (Previously Presented): A type I polyketide synthase according to claim 56, wherein said acyltransferase domain is the acyltransferase of module 6 of the niddamycin polyketide synthase.

Claim 62 (Previously Presented): A type I polyketide synthase according to claim 56, wherein said acyltransferase domain is the acyltransferase of module 4 of the FK506 polyketide synthase.

Claim 63 (Currently Amended): A type I polyketide synthase according to claim 54, wherein said polyketide synthase is effective to synthesize a polyketide selected from

- (a) 12- and 16-membered macrolides with acetate starter units;
- (b) 12, 14, and 16-membered macrolides with propionate starter units;
- (c) variants of rifamycin, avermectin, rapamycin, immunomycin and FK506 which differ from the natural compound in the incorporation of acetate starter units or propionate starter units;
- (d) a polyketide wherein the starter unit is derived by the action of said engineered-KS<sub>Q</sub> domain on the enzyme-bound product of from a loading domain comprising the acyltransferase said AT domain, wherein said AT domain is from extension module 4 of the FK506 polyketide synthase; or
- (e) a polyketide wherein the starter unit is derived by the action of said engineered-KS<sub>Q</sub> domain on the enzyme-bound product of from a loading domain comprising the acyltransferase said AT domain, wherein said AT domain is from extension module 6 of the niddamycin polyketide synthase.

Claim 64 (Currently Amended): A type I polyketide synthase which produces a polyketide and which comprises a loading module and a plurality of extension modules, wherein:

- a) said loading module loads an optionally substituted malonyl and then effects decarboxylation of the loaded moiety to provide a corresponding optionally substituted acetyl moiety for transfer to the first of said extension modules; and
- b) said loading module is of the form:  
(KS<sub>Q</sub>) - (AT) - (ACP), wherein:
  - i) ACP is an acyl carrier protein domain;
  - ii) AT is an acyltransferase domain which loads an optionally substituted malonyl and is selected from the group

consisting of the acyltransferase domain of module 6 of the niddamycin polyketide synthase, the acyltransferase domain of module 4 of the FK506 polyketide synthase, and the acyltransferase domain of module 2 of the rapamycin polyketide synthase; the acyltransferase domain of module 5 of the spiramycin polyketide synthase, and the acyltransferase domain of module 5 of the monensin polyketide synthase; and

iii) KSq is a domain which effects decarboxylation of a loaded optionally substituted malonyl and which differs from a ketosynthase domain of an extension module by having a glutamine residue in place of the cysteine residue in the active site; and

e) at least the first of said extension modules is not naturally associated with said leading module;

wherein the polyketide produced by the polyketide synthase is other than a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

Claim 65 (Previously Presented): The type I polyketide synthase according to claim 64, wherein (AT) is the acyltransferase domain of module 6 of the niddamycin polyketide synthase,

Claim 66 (Previously Presented): The type I polyketide synthase according to claim 64, wherein (AT) is the acyltransferase domain of module 4 of the FK506 polyketide synthase.

Claim 67 (Cancelled)

Claim 68 (Previously Presented): The type I polyketide synthase according to claim 64, wherein (AT) is the acyltransferase domain of module 5 of the spiramycin polyketide synthase.

Claim 69 (Cancelled)

Claim 70 (Currently Amended) : A type I polyketide synthase which produces a polyketide and which comprises a loading module and a plurality of extension modules, wherein said loading module is of the form:

(KSq) - (AT) - (ACP), wherein:

- i) ACP is the acyl carrier protein domain of the erythromycin loading module;
- ii) AT is the acyltransferase domain of module 2 of the rapamycin polyketide synthase; and
- iii) The type I polyketide synthase according to claim 64, wherein the KSq domain is the KSq domain of the oleandomycin loading module.

Claim 71 (Currently Amended) : The type I polyketide synthase according to claim 64, wherein said extension modules are selected from the group consisting of extension modules from the erythromycin, rifamycin, avermectin, rapamycin, immunomycin, or FK506 polyketide synthases.

Claim 72 (Cancelled)

Claim 73 (Currently Amended) : A type I polyketide synthase which produces a polyketide and which comprises a loading module and a plurality of extension modules, wherein:

- a) said loading module leads an optionally substituted malonyl and then effects decarboxylation of the loaded moiety to provide a corresponding optionally substituted acetyl moiety for transfer to the first of said extension modules;
- b) said loading module is of the form:

(KSq) - (AT) - (ACP), wherein:

- i) ACP is an acyl carrier protein domain;
- ii) AT is an acyltransferase domain which leads an

~~optionally substituted malonyl, and~~

~~iii) KSq is a domain which effects decarboxylation of a loaded optionally substituted malonyl and which differs from a ketosynthase domain of an extension module by having a glutamine residue in place of the cysteine residue in the active site;~~

~~e) at least the first of said extension modules is not naturally associated with said loading module; and~~

~~d) said loading module is the loading module of the monensin polyketide synthase; and~~

~~b) at least the first of said extension modules is not naturally associated with said loading module;~~

wherein the polyketide produced by the polyketide synthase is other than a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

Claim 74 (Cancelled)

Claim 75 (Currently Amended): A type I polyketide synthase which produces a 12- or 14- membered macrolide and which comprises a loading module and a plurality of extension modules, wherein:

a) said loading module ~~leads an optionally substituted malonyl and then effects decarboxylation of the loaded moiety to provide a corresponding optionally substituted acetyl moiety for transfer to the first of said extension modules;~~

b) said loading module is of the form:

~~(KSq) (AT) (ACP), wherein-~~

~~i) ACP is an acyl carrier protein domain;~~

~~ii) AT is an acyltransferase domain which loads an optionally substituted malonyl; and~~

~~iii) KSq is a domain which effects decarboxylation of a loaded optionally substituted malonyl and which differs from a ketosynthase domain of an extension module by having a~~

~~glutamine residue in place of the cysteine residue in the active site;~~

~~e) at least the first of said extension modules is not naturally associated with said loading module; and~~

~~d) said loading module is the loading module of the tylosin polyketide synthase; and~~

~~b) at least the first of said extension modules is not naturally associated with said loading module;~~

wherein the polyketide produced by the polyketide synthase is other than a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

Claim 76 (Cancelled)